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TITLE: SAFETY AND IMMUNOGENICITY OF AN INACTIVATED WHOLE CELL PLUS RECOMBINANT B SUBUNIT (WC/RBS) COLERA VACCINE IN HEALTHY ADULT PERUVIAN MILITARY VOLUNTEERS

SUBTITLE: Results of a Phase II Study of Whole Cell/Recombinant B-Subunit (WC/rBS) Oral Cholera Vaccine in Peruvian Adults

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Subject: MIPR No. 92MM2532, RESULTS OF A PHASE II STUDY OF WHOLE CELL/RECOMBINANT B-SUBUNIT (WC/rBS) ORAL CHOLERA VACCINE IN PERUVIAN ADULTS.

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Design:

To evaluate both safety and immunogenicity of the WC/rBS killed vaccine in healthy Peruvian adults the collaborative team conducted a randomized, double-blinded, placebo-controlled study at the Ancon Peruvian Marine Corps Base north of Lima.

Study Population and Conduct of Study:

Volunteers were solicited from incoming recruit companies. Four companies were requested to participate in the study, and the base staff, both line and medical, approved of the participation of recruits. All were of Hispanic or Indian-Hispanic ethnicity, from lower to lower-middle socioeconomic status. All were males. Each participant was screened prior to entry into the study via questionnaire and oral temperature examination. Anyone with a history of cholera was not entered. None had ever received cholera vaccination through enteral or parenteral routes. Those admitting recent illness, fever, recent use of antibiotics or antacids, history of chronic gastrointestinal problems, or diarrhea in the previous five days were ineligible. Three hundred forty six (346) recruits from four Marine Corps companies were entered, 175 randomized to receive 3 ml of the vaccine mixed with 150 cc of a prepared buffer solution, 171 to receive the buffer

(control) solution alone. The buffer solution was prepared prior to administering vaccine to each company of recruits using distilled water. Administration of vaccine and the placebo (control preparation consisting only of buffer) was in partially opaque plastic disposable cups to avoid identification of any slight physical differences, and the consumption of the entire dosage was witnessed prior to their departure from the administration area.

Blood samples were obtained using sterile technique from each recruit prior to the first dose. These were analyzed by NAMRID, with second aliquots sent to WRAIR, and a third to the University of Goteborg. Administration of vaccination and control preparation was carried out on Monday, 10 February, and Tuesday, 11 February,1992. For one month prior to the first administration day an on-base diarrheal surveillance system was established and used to monitor bacterial causes of diarrhea. On the first day of vaccination NAMRID's Microbiology department identified three cases of diarrhea caused by Vibrio cholerae, 01, El Tor, Ogawa in samples obtained from recruits sampled five days before the first vaccination. Review of the situtation with the Medical Monitor for the study and the Human Use Committee cleared the continuation of the study as cases of cholera had been identified as introducted prior to the administration of the first doses of vaccine. Had this not been the case we would have been bound to terminate the study at this point. Second doses of vaccine/ control preparation were administered to each recruit who had received the initial doses fourteen days after their first dose. Blood samples were again obtained from 307 remaining recruits two weeks after the second vaccine/control preparation administration, and aliquots of serum similarly distributed as was the case with the first samples. For each of three days after vaccinations every recruit was interviewed for side effect including diarrhea, abdominal cramping, gas, headache, fever, and weakness. The number of formed, unformed and liquid stools was noted for each twenty four hour period, and the degree of severity of side effects was classified. Mild symptoms were those present when the volunteer thought about it, moderate symptoms were present constantly without interfering with daily activities, and severe symptoms interfered with activities. Anyone with diarhea, defined as three or more loose, unformed, or liquid stools in any twenty four hour period was sent to medical for a stool culture. Anyone with symptoms self-classified as severe was evaluated by a physician. Subsequent to the three initial cases of cholera additional cases were found. Peak numbers of diarrhea were reported after each of the vaccination days in both vaccine and control preparation recipients. Environmental sampling and a case control study could not identify a definitive source of the outbreak. Suspected sources were introduction of cholera organisms into the cistern used for potable water exclusively by recruits, possibly by those who were asymptomatic, and contaminated food provided to recruits by visiting family members on weekly visits. The practice of

allowing foods to be given to recruits was subsequently prohibited. A previous outbreak investigation of more than 130 recruits at the same base in spring of 1991 found a potable water cistern used exclusively by recruits was contaminated with Vibrio cholerae, 01, El Tor, Inaba. The changovever from Inaba to Ogawa is one of the notable changes in the epidemiology of cholera in Peru, and is consistent with the reports of collaborating institutions in Peru. With the three cases of microbiologically-confirmed cholera we chose to evaluate, through the use of rectal swabs, the number of symptomatic and asymptomatic cases which were present in the study population. For logistic reasons it was only possible to evaluate approximately half of the recruit companies participating in the study. On 17 February 216 recruits had a rectal culture done. Thirty seven recruits were positive for Vibrio cholerae, 01, El Tor, Ogawa. One recruit was positive for Vibrio cholerae, 01, El Tor, Inaba. Thus 38/216 (17.6%) were culturable for viable cholera organisms. Only nine of the thirty-eight stated they had any diarrhea during the study. Being restricted to a single sampling between the two vaccinations we no doubt misses those who had already stopped excretion of viable organisms, those who were not yet infected, and a certain number who might have been detected on stool rather than rectal cultures. Subsequent interviews of fifty recruits found that despite standing orders to go to medical if they had diarrhea less than 15% actually did so. Reasons included having self-treated despite direction not to do so, not considering the issue a significant problem, and the emphasis of their chain of command to avoid time away from their military training requirements. Due to the impact of the epidemic on our study population we restricted our analysis to the recruits who were screened by rectal swab and were found to be culture negative for cholera, and who were not found to be infected with cholera at any time during the study.

Safety of the Vaccine

Comparison of vaccine and control preparation recipients showed there to be no significant differences in the incidence of side effects, with most reporting mild to moderate symptoms. The most common complaints were diarrhea and abdominal cramps. Evaluating all recruits who entered the study, rates of diarrhea among control recipients and vaccinees on their first day of vaccination were 5.8% and 5.1% respectively, on day two post-vaccination 6.4% and 8.0%, and day three post-vaccination 8.2% and 7.4%. Rates of other side effects were lower and similar among both groups.

Immunologic Response:

Setting the baseline for non-exposure to cholera at a Vibriocidal titer of zero on the pre-vaccination blood samples would leave only twenty four recipients. Among vaccine recipients an

anticholera toxin antibody increase (0.2 or greater rise) was statistically significant with an odds ratio of 7.20 with 0.035 P value. Using a less strict basal vibriocidal titer of less than 1:80 as consistent with non-exposure leaves fifty two evaluable volunteers. These men showed a marked anticholera toxin antiboy increase with an odds ratio of 33.00 among recipients of vaccine, p value <<.01 . In contrast, geometric mean fold increase between vaccine and control preparation recipientsdid not show a significant difference. Baseline titers to the <80 level could indicate exposure to non-cholera vibrios, exposure to other cross-reacting substances, the residual of exposure to the epidemics of 1991, 1992, or asymptomatic or mildly symptomatic cases, or laboratory variation. Testing by the University of Goteborg has also found a large percentage of elevated pre-vaccination antibody levels, and we await opportunity to correlate our laboratory results with them and with WRAIR. As a result of finding the elevated pre-vaccination levels NAMRID intends to review our serum banks to determine levels among similar populations from before the late January, 1991 outbreak of cholera along the coast in Peru.

Summary:

The Whole Cell Recombinant B-Subunit Oral Cholera Vaccine is safe in this population. In this study there was not significant elevation of vibriocidal antibody level in vaccine recipients, but vaccination did raise anti-cholera toxin antibodies significantly among those with low levels prior to vaccination with the two dose vaccination regimen. The clinical significance, including vaccine efficacy, of these results for South American populations remains to be evaluated in a Phase III study.

First Dose:

Tables 1 - 4 Analysis of side effects restricting observations to those with baseline Vibriocidal Titer = 0

Table 1 Any Side Effect

| | + | - | Total |
|---|---|---|---|
| Vaccine | 10 | 0 | 10 |
| Control | 13 | 1 | 14 |
| Total | 23 | 1 | 24 |

Odds Ratio = 1.08 with P Value = 0.398

Table 2 Severe Side Effect

| | + | - | Total |
|---|---|---|---|
| Vaccine | 1 | 9 | 10 |
| Control | 3 | 11 | 14 |
| Total | 4 | 20 | 24 |

Odds Ratio = 0.41 with P Value = 0.468

Table 3 Diarrhea

| | + | - | Total |
|---|---|---|---|
| Vaccine | 1 | 9 | 10 |
| Control | 0 | 14 | 14 |
| Total | 1 | 23 | 24 |

Mantel-Haenszel P Value = 0.236

Table 4 Any Cramps

| | + | - | Total |
|---|---|---|---|
| Vaccine | 3 | 7 | 10 |
| Control | 7 | 7 | 14 |
| Total | 10 | 14 | 24 |

Odds Ratio = 0.43 with Mantel-Haenszel P Value = 0.337

Following first dose table analysis includes those with Vibriocidal titer at baseline <80. Tables 5 - 8.

Table 5 Any Side Effect

| | + | - | Total |
|---|---|---|---|
| Vaccine | 23 | 1 | 24 |
| Control | 25 | 3 | 28 |
| Total | 48 | 4 | 52 |

Odds Ratio = 2.76 with P Value = 0.381

Table 6 Severe Side Effect

| | + | - | Total |
|---|---|---|---|
| Vaccine | 2 | 22 | 24 |
| Control | 4 | 24 | 28 |
| Total | 6 | 46 | 52 |

Odds Ratio = 0.55 with P Value = 0.507

Table 7 Diarrhea

| | + | - | Total |
|---|---|---|---|
| Vaccine | 2 | 22 | 24 |
| Control | 1 | 27 | 28 |
| Total | 3 | 49 | 52 |

Odds Ratio = 2.45 with Mantel-Haenszel P Value = 0.467

Table 8 Any Cramps

| | + | - | Total |
|---|---|---|---|
| Vaccine | 8 | 16 | 24 |
| Control | 14 | 14 | 28 |
| Total | 22 | 30 | 52 |

Odds Ratio = 0.50 with Mantel-Haenszel P Value = 0.229

Second Dose:

Table 9 - 12 are analysis of second dose recipients with tables restricted to those with baseline Vibriocidal titer = 0 .

| Table 9 | Any Side Effect | | |
|---|---|---|---|
| | + | - | Total |
| Vaccine | 10 | 0 | 10 |
| Control | 10 | 1 | 11 |
| Total | 20 | 1 | 21 |

Odds Ratio = 1.10 with P Value = 0.340

| Table 10 | Severe Side Effect | | |
|---|---|---|---|
| | + | - | Total |
| Vaccine | 0 | 10 | 10 |
| Control | 1 | 10 | 11 |
| Total | 1 | 20 | 21 |

Odds Ratio = 0.0 with P Value = 0.340

| Table 11 | Diarrhea | | |
|---|---|---|---|
| | + | - | Total |
| Vaccine | 1 | 9 | 10 |
| Control | 0 | 11 | 11 |
| Total | 1 | 20 | 21 |

Mantel-Haenszel P Value = 0.294

| Table 12 | Any Cramps | | |
|---|---|---|---|
| | + | - | Total |
| Vaccine | 4 | 6 | 10 |
| Control | 5 | 6 | 11 |
| Total | 9 | 12 | 21 |

Odds Ratio = 0.80 with Mantel-Haenszel P Value = 0.805

Table 13 - 16 are analysis of second dose recipients whose Vibriocidal baseline titer < 80.

| Table 13 | Any Side Effect | | |
|---|---|---|---|
| | + | - | Total |
| Vaccine | 19 | 3 | 22 |
| Control | 23 | 1 | 24 |
| Total | 42 | 4 | 46 |

Odds Ratio = 0.28 with P Value = 0.26

| Table 14 | Severe Side Effect | | |
|---|---|---|---|
| | + | - | Total |
| Vaccine | 0 | 22 | 22 |
| Control | 2 | 22 | 24 |
| Total | 2 | 44 | 46 |

Odds Ratio = 0.0 with P Value = 0.17

Table 15 Diarrhea

| | + | - | Total |
|---|---|---|---|
| Vaccine | 3 | 19 | 22 |
| Control | 3 | 21 | 24 |
| Total | 6 | 40 | 46 |

Odds Ratio = 1.11 with Mantel-Haenszel P Value = 0.9

Table 16 Any Cramps

| | + | - | Total |
|---|---|---|---|
| Vaccine | 6 | 16 | 22 |
| Control | 10 | 14 | 24 |
| Total | 16 | 30 | 46 |

Tables 17 - 19 show lack of significant response in elevation of mean fold titer of Vibriocidal antibody and significant anticholera toxin increases in the study population.

Table 17 Vibriocidal Antibody Response (Original Titer < 80)

| | Observations | Mean Fold | Median Fold |
|---|---|---|---|
| Vaccine | 24 | 3.75 | 3.00 |
| Control | 28 | 3.53 | 2.00 |

Table 18 Anticholera Toxin Increase (Original Vibriocidal Titer= 0)

| | + | - | Total |
|---|---|---|---|
| Vaccine | 8 | 2 | 10 |
| Control | 5 | 9 | 14 |
| Total | 13 | 11 | 24 |

Odds Ratio = 7.20 with P Value = 0.035

Table 19 Anticholera Toxin Increase (Original Vibriocidal Titer = < 80)

| | + | - | Total |
|---|---|---|---|
| Vaccine | 22 | 2 | 24 |
| Control | 7 | 21 | 28 |
| Total | 29 | 23 | 52 |

Odds Ratio = 33.0 with P Value = .00000176